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THE OPSONIC INDEX IN DIPHTHERIA.*

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ACCORDING to Wright¹ diphtheria bacilli are insensible to the opsonic action of blood fluids. This conclusion is based upon experiments showing that there is as much (or, in one case, more) phagocytosis in the presence of normal human serum heated at 60° C. as in unheated. In order to study this question four strains of typical diphtheria bacilli furnished me by Dr. Hamilton were tested. With all of these strains there was no opsonin demonstrable in normal human serum after heating the serum 30 minutes at 50° C. This was found to be true also of serum from diphtheria patients when tested with strain "N," the organism employed in the subsequent experiments.

Reque² also found that when washed leucocytes are mixed with normal human or dog serum heated to 58–60° for 15–30 minutes, there is practically no phagocytosis with diphtheria bacilli. It is possible that the strain which Wright used was subject to the spontaneous phagocytosis which has been observed to occur with occasional strains of diphtheria bacilli.

As the course of the specific opsonic index in certain acute infections has been found to possess characteristic features that harmonize well with the general clinical picture, rising above normal as the symptoms decline, it was thought it would be of interest to determine if this were also true in regard to diphtheria. Both the diphtheria- and the streptococco-opsonic indices were estimated on account of the close association of streptococci with tonsillar infections.

The Wright method of estimating the opsonic index has been employed for the most part. The suspensions are made from 24-, occasionally 48-hour, cultures on plain agar or Löffler's blood serum. The fluid of condensation is discarded on account of its containing clumps. A suspension is made in enough 0.85 per cent salt solution

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¹ *Proc. Royal Soc.*, 1904, 73, p. 128.

² *Jour. Infect. Dis.*, 1906, 3, p. 441.

to give an average count of 1.5 diphtheria bacilli (the suspension containing about 300,000 bacilli per c.mm.) and 3-5 streptococci per leucocyte in the control specimen with normal serum. The Wright method of determining the opsonic index has been criticized on account of inaccuracy due to variation in the thickness of the bacterial suspensions. Suspensions of diphtheria bacilli varying from 125,000 to 1,000,000 bacilli per c.mm. were found, however, to give practically the same indices, as seen from the table:

TABLE 1

Suspension (No. bacilli per c.mm.)	Opsonic Index
1,000,000.....	1.16
750,000.....	1.16
600,000.....	1.2
500,000.....	1.15
500,000.....	1.2
450,000.....	1.5
375,000.....	1.2
300,000.....	1.3
250,000.....	1.2
250,000.....	1.2
150,000.....	1.3
125,000.....	1.2

The serum from three normal individuals is pooled and used as the normal control. The pool and the patient's serum are collected within an hour of each other and removed from the clot at nearly the same time because of the difference in the quantity of opsonin in serum removed at different times after clotting. The washed blood (leucocytes) is obtained from an individual whose red blood cells are not susceptible to isoagglutination. Fifty polymorphonuclear neutrophils are counted, 25 at each end of the slide. If these two counts do not closely correspond, more leucocytes are counted. Clumps of bacteria as well as clumps of leucocytes are discarded. Isolated or paired leucocytes only are counted.

In my preliminary tests I found that the diphtheria index in normal adult persons may vary between 0.92 and 1.1, except that in one case it was 0.8 and in another 1.2. The normal streptococcal index I found to vary from 0.9 to 1.1.

As all of the patients examined received diphtheria antitoxin, it seemed advisable to determine if this injection had any immediate influence upon the opsonic index for diphtheria bacilli. A normal man was injected with 12,000 units and his index taken several days

before and after the injection, but at no time was it found outside the normal limit, varying from 0.92-1.1.

The diphtheria- and streptococco-opsonic indices of 14 diphtheria

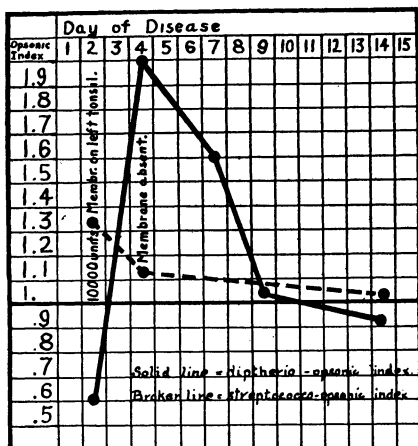


CHART 1.—Opsonic indices for *B. diphtheriae* and streptococcus in diphtheria (man, 30 years, no fever).

patients have been observed as a rule daily throughout the course of the disease. The diagnosis of diphtheria was confirmed in each case by bacteriological examination, except in two laryngeal cases in which the cultures were negative. The cases occurred at the Cook County Hospital, in the services of Dr. W. L. Baum and Dr. Geo. H. Weaver, to whom I am indebted for the privilege of making the examinations.

In eight cases, all examined early in the disease, the diphtheria index was found at first to be below normal, ranging between 0.4 and 0.7. Of the six cases in

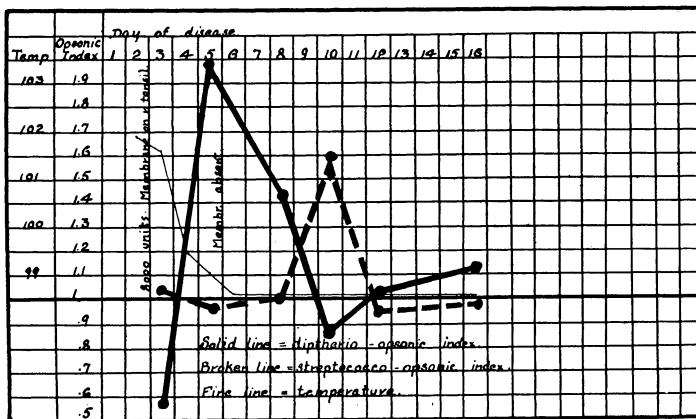


CHART 2.—Opsonic indices for *B. diphtheriae* and streptococcus in diphtheria (man, 36 years).

which no negative phase was observed, two were first examined after the membrane had disappeared and three were laryngeal cases.

In all of the patients the disappearance of the membrane and improvement in the symptoms were accompanied with a rise above normal of the indices which varied from 1.3 to 2.2, the average

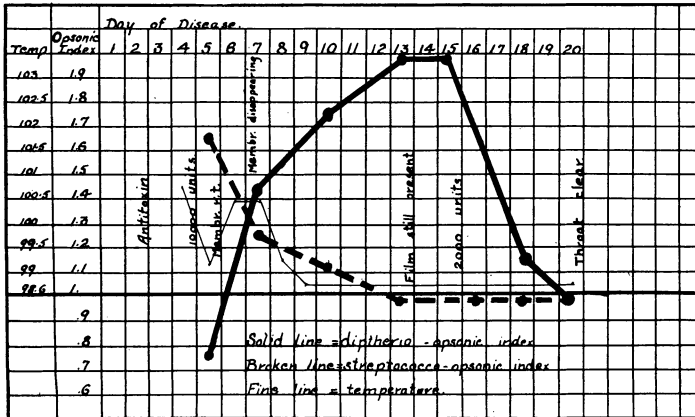


CHART 3.—Opsonic indices for *B. diphtheriae* and streptococcus in diphtheria (girl, 10 years).

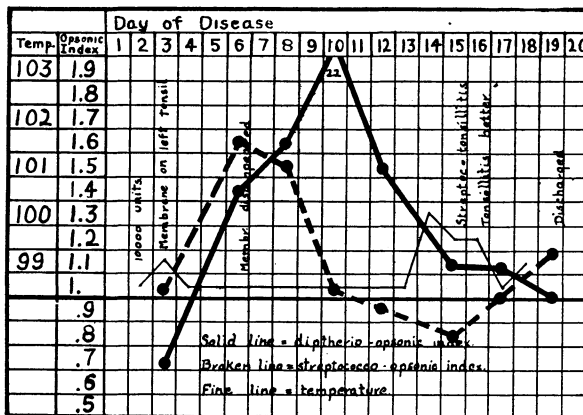


CHART 4.—Opsonic indices for *B. diphtheriae* and streptococcus in diphtheria (woman, 25 years).

highest point being 1.9. This rise was followed by a fall to normal in from 2-9 days (see Charts 1, 2, 3, 4).

In two cases the streptococcal index remained normal. The index was found below normal only twice. In the majority of cases the rise in this index occurred earlier in the attack than that of the

diphtheria index. The two indices corresponded in only one case. This independence in the course of these two indices would seem to indicate that the opsonins in question are specific.

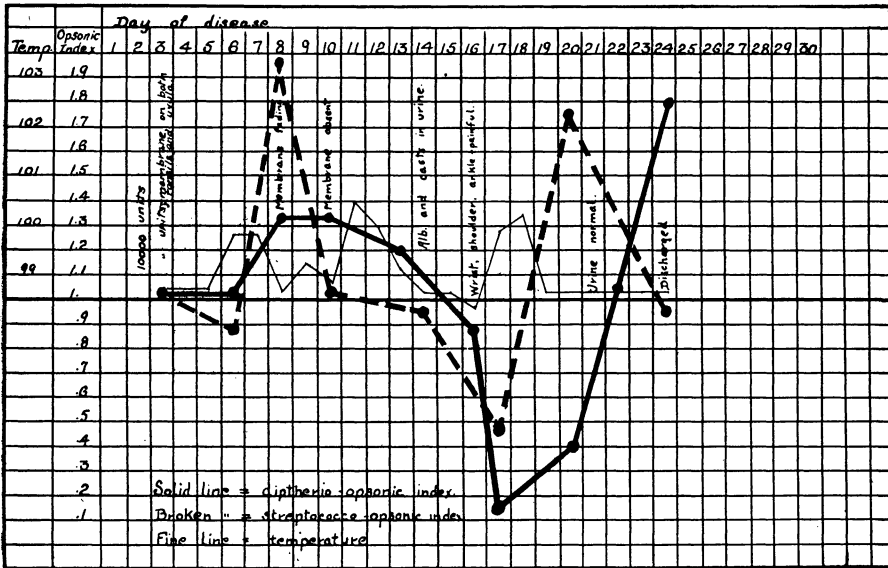


CHART 5.—Opsonic indices for *B. diphtheriae* and streptococcus in diphtheria complicated by nephritis and arthritis (man, 25 years).

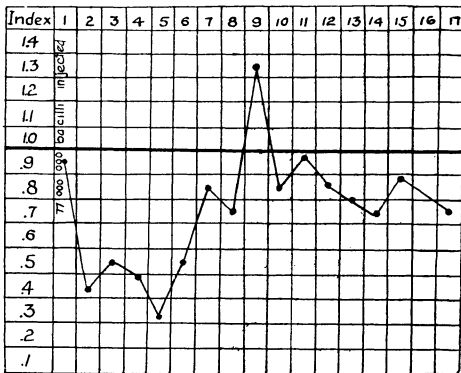


CHART 6.—Diphtheria-opsonic index in rabbit injected with killed diphtheria bacilli.

In the one complicated case (Chart 5) examined, there were observed with the onset of nephritis and arthritis, low indices followed by decided rises as improvement occurred. This corresponds to the observations made by Banks¹ and myself² in cases of scarlet fever associated with complications.

Reque made a few examinations in two cases of diphtheria and found a decrease in opsonin for diphtheria bacilli on the 7th and 14th

¹ Jour. Path. and Bact., 1907, 12, p. 113.

² Jour. Infect. Dis., 1907, 4, p. 304.

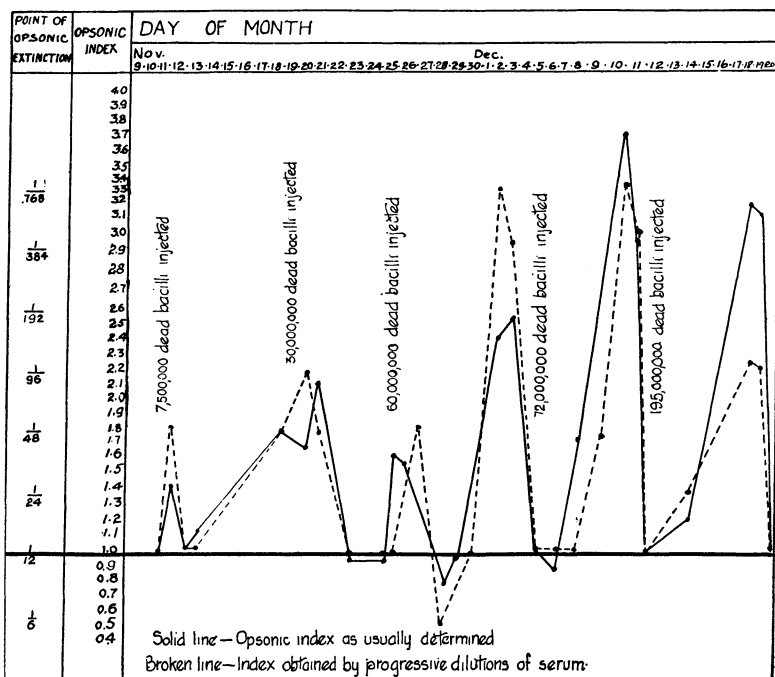


CHART 7.—Comparison of opsonic index as usually determined and index obtained by progressive dilutions of the serum in rabbit injected with killed diphtheria bacilli.

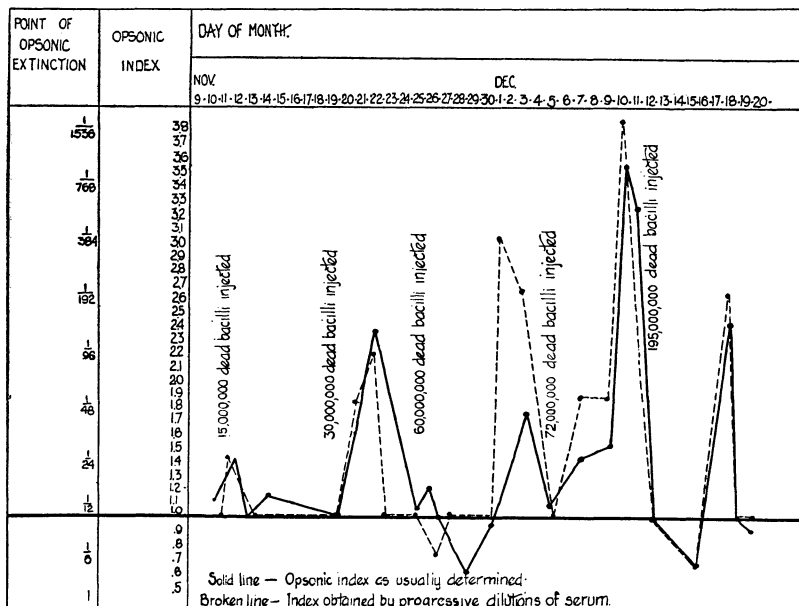


CHART 8.—Comparison of opsonic index as usually determined and index obtained by progressive dilutions of the serum in rabbit infected with killed diphtheria bacilli.

days and an increase during convalescence. Although in the majority of my cases the low and high indices occurred earlier than his, our observations on the whole agree.

Rabbits were injected with dead diphtheria bacilli to determine whether or not the opsonic indices would correspond to the course

of the index in cases of diphtheria.

The first injection of 17,000,000 dead bacilli produced a prolonged negative phase, followed by only a slight rise on one day, the 9th day after injection (Chart 6). Charts 7 and 8 show the effect of repeated injections of a smaller number of organisms. Each injection was followed by a rise in the index. In only the one case was a negative phase demonstrated, namely after injection of 72,000,000 organisms, showing apparently that large doses only are likely to produce negative phases in rabbits.

In five cases of diphtheria, the mixtures of serum, leucocytes, and suspension were incubated at 45° C. for five minutes. Chart 9 shows that the indices differ in height, when incubated at 37° and 45° C.; otherwise they correspond fairly closely in their course. Five minutes was chosen for the time of incubation

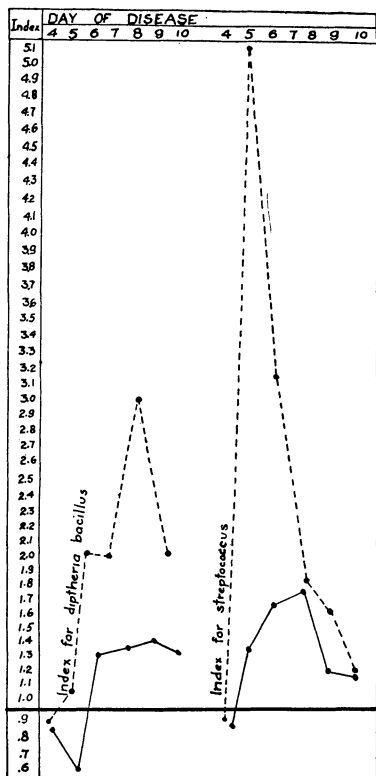


CHART 9.—Opsonic indices for *B. diphtheriae* and streptococcus in diphtheria (woman, 30 years) as obtained after incubation of mixtures at 37° C. for five minutes (solid line) and after incubation at 45° C. for five minutes (broken line).

on account of the rapid destruction of the leucocytes at 45° C.

Klien¹ observes that the opsonic index in rabbits injected with typhoid bacilli, as estimated by the Wright method, does not show the real amount of opsonin present. He therefore diluted the normal and

¹ Bull. Johns Hopkins Hosp., 1907, 18, p. 245.

immune serum with normal salt solution to the point where phagocytosis recognizably exceeded spontaneous phagocytosis and in this way measured the opsonic power. It was thought well to employ this method in connection with the present investigation. As there was a slight spontaneous phagocytosis with the diphtheria bacillus employed, a control test with salt solution was always made. I found that with a suspension of about 300,000 bacilli per c.mm. normal opsonin in

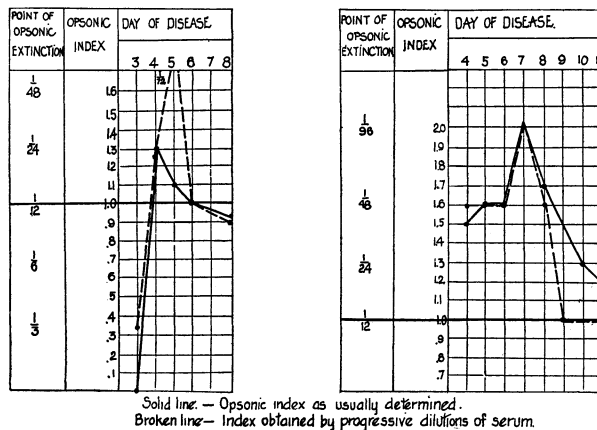


CHART 10.—Comparison of opsonic index for *B. diphtheriae* as usually determined and index obtained by progressive dilutions of the serum in a mild, uncomplicated case of diphtheria.

human and rabbit serum disappeared at a dilution of 1-24, there always being some present at 1-12. The percentage and the phagocytic indices were found to closely agree, calculating the percentage index taking much less time. The opsonic indices of two diphtheria patients and two rabbits injected with dead diphtheria bacilli were estimated in the usual way, at the same time as the opsonic power was calculated by diluting the serum to the point just exceeding spontaneous phagocytosis. As seen in Charts 7 and 8, the two methods give in this case results which correspond very closely.

Dean¹ found that there was no direct proportion between the serum concentration and the number of organisms ingested, there being as many or more with the half, quarter, or even one-eighth concentration as with the full strength. He tested staphylococci and tubercle bacilli with serum of several species of animals. In 85 experiments

¹ *Proc. Royal Soc.*, 1907, B. 79, p. 399.

with the diphtheria bacillus, using both human and rabbit serum, only once did I find as many organisms taken up in the half-strength as in the full.

CONCLUSIONS.

The diphtheria bacillus is sensible to the opsonic action of human and rabbit blood serum.

In diphtheria the opsonic index for diphtheria bacilli is generally below normal at the onset of the disease. As the membrane disappears and the symptoms subside the index rises considerably, returning to normal in from two to nine days.

In the majority of cases there is a rise in the streptococco-opsonic index in diphtheria. The indices for diphtheria bacilli and streptococci rarely correspond.

The injection of dead diphtheria bacilli in suitable number into rabbits is followed by a rise in the index.

The indices obtained after incubating the mixtures at 45° C. are higher but correspond fairly closely with the course of the indices obtained after incubation of the mixtures at 37° C.

By diluting the normal and the immune serum until phagocytosis practically ceases, the comparative opsonic power of the sera on diphtheria bacilli can be measured. The curves representing the opsonic power determined in this way correspond almost exactly with the curves of the opsonic indices estimated by Wright's method.

The suggestion may be permitted that the injection of dead diphtheria bacilli may prove of some service in ridding the throat of bacilli in the case of chronic carriers and convalescents, as the experiments show that in rabbits such injections are harmless and at the same time cause decided increase in the opsonic power of the blood upon diphtheria bacilli.